

11/16/00  
JC923 U.S. PTO

PTO/SB/05 (08-00)  
Approved for use through 10/31/2002. OMB 0651-0032  
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# UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.

First Inventor

Nathan C. Maier

Title

The use of mosquito salivary  
tachykinins to remediate...

Express Mail Label No.

## APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☒ Fee Transmittal Form (e.g., PTO/SB/17)  
(Submit an original and a duplicate for fee processing)
2. ☒ Applicant claims small entity status.  
See 37 CFR 1.27.
3. ☒ Specification [Total Pages ☒ 9 ]  
(preferred arrangement set forth below)
  - Descriptive title of the invention
  - Cross Reference to Related Applications
  - Statement Regarding Fed sponsored R & D
  - Reference to sequence listing, a table, or a computer program listing appendix
  - Background of the Invention
  - Brief Summary of the Invention
  - Brief Description of the Drawings (if filed)
  - Detailed Description
  - Claim(s)
  - Abstract of the Disclosure
4. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets ☒ 1 ]
5. Oath or Declaration [Total Pages ☒ 2 ]
  - a. ☒ Newly executed (original or copy)
  - b. ☐ Copy from a prior application (37 CFR 1.63 (d))  
(for continuation/divisional with Box 17 completed)
  - i. ☐ **DELETION OF INVENTOR(S)**  
Signed statement attached deleting inventor(s)  
named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☐ Application Data Sheet. See 37 CFR 1.76

## ADDRESS TO:

Assistant Commissioner for Patents  
Box Patent Application  
Washington, DC 20231

7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program (Appendix)
8. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
  - a. ☐ Computer Readable Form (CRF)
  - b. Specification Sequence Listing on:
    - i. ☐ CD-ROM or CD-R (2 copies); or
    - ii. ☐ paper
  - c. ☐ Statements verifying identity of above copies

## ACCOMPANYING APPLICATION PARTS

9. ☐ Assignment Papers (cover sheet & document(s))
10. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney  
(when there is an assignee)
11. ☐ English Translation Document (if applicable)
12. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
13. ☐ Preliminary Amendment
14. ☒ Return Receipt Postcard (MPEP 503)  
(Should be specifically itemized)
15. ☐ Certified Copy of Priority Document(s)  
(if foreign priority is claimed)
16. ☐ Other: .....

17. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP)

of prior application No. \_\_\_\_\_

Prior application information

Examiner \_\_\_\_\_

Group / Art Unit \_\_\_\_\_

For CONTINUATION OR DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

## 18. CORRESPONDENCE ADDRESS

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or ☒ Correspondence address below

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Name (Print/Type)	Nathan C. Maier	Registration No. (Attorney/Agent)	
Signature	Nathan Maier	Date	Nov. 14/00

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09/713299

# FEE TRANSMITTAL for FY 2001

*Patent fees are subject to annual revision.*

**TOTAL AMOUNT OF PAYMENT**

**(\$)** 435.00

## Complete if Known

Application Number	
Filing Date	
First Named Inventor	Nathan C. Maier
Examiner Name	
Group Art Unit	
Attorney Docket No.	

## METHOD OF PAYMENT

1. ☐ The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:

Deposit Account Number   
Deposit Account Name

☐ Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17

☒ Applicant claims small entity status. See 37 CFR 1.27

2. ☒ **Payment Enclosed:**

☐ Check ☐ Credit card ☒ Money Order ☐ Other

## FEE CALCULATION

### 1. BASIC FILING FEE

Large Entity		Small Entity		Fee Description	Fee Paid
Fee Code	Fee (\$)	Fee Code	Fee (\$)		
101	710	201	355	Utility filing fee	355
106	320	206	160	Design filing fee	
107	490	207	245	Plant filing fee	
108	710	208	355	Reissue filing fee	
114	150	214	75	Provisional filing fee	

**SUBTOTAL (1)** (\$ ) 355

### 2. EXTRA CLAIM FEES

Total Claims		Extra Claims		Fee from below		Fee Paid	
Independent Claims	5	-20** =	0	X		=	
Multiple Dependent Claims	5	-3** =	2	X	40	=	80

Large Entity		Small Entity		Fee Description
Fee Code	Fee (\$)	Fee Code	Fee (\$)	
103	18	203	9	Claims in excess of 20
102	80	202	40	Independent claims in excess of 3
104	270	204	135	Multiple dependent claim, if not paid
109	80	209	40	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

**SUBTOTAL (2)** (\$ ) 80

\*\*or number previously paid, if greater; For Reissues, see above

## FEE CALCULATION (continued)

### 3. ADDITIONAL FEES

Large Entity		Small Entity		Fee Description	Fee Paid
Fee Code	Fee (\$)	Fee Code	Fee (\$)		
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for <i>ex parte</i> reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	390	216	195	Extension for reply within second month	
117	890	217	445	Extension for reply within third month	
118	1,390	218	695	Extension for reply within fourth month	
128	1,890	228	945	Extension for reply within fifth month	
119	310	219	155	Notice of Appeal	
120	310	220	155	Filing a brief in support of an appeal	
121	270	221	135	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,240	241	620	Petition to revive - unintentional	
142	1,240	242	620	Utility issue fee (or reissue)	
143	440	243	220	Design issue fee	
144	600	244	300	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	710	246	355	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	710	249	355	For each additional invention to be examined (37 CFR § 1.129(b))	
179	710	279	355	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) \_\_\_\_\_

\* Reduced by Basic Filing Fee Paid

**SUBTOTAL (3)** (\$ )

## SUBMITTED BY

Name (Print/Type)	Nathan C. Maier	Registration No. (Attorney/Agent)	Complete (if applicable)
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			Date Nov. 14/00

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## INTRODUCTION:

Title of invention: The use of mosquito salivary tachykinins to remediate unregulated cellular proliferation.

### Inventors:

Nathan C. Maier, Canadian citizen, and resident of Longview, TX.

Amiel G. Jarstfer, United States citizen, and resident of Longview, TX.

## REFERENCES CITED:

### U.S. Patent Documents

<u>5990125</u>	Nov., 1999	Howard	514/305
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### Other References

Champagne, D. E. and J. M. C. Ribeiro (1994) Sialokinin I and II: Vasodilatory tachykinins from the yellow fever mosquito *Aedes aegypti*. *Proc. Natl. Acad. Sci.* **91**, 138-142.

Flaitz, C. M. and M. J. Hicks (1998) Molecular piracy: the viral link to carcinogenesis. *Oral Oncology*. **34**, 448-453.

Hedge, R. S. and E. J. Androphy (1998) Crystal structure of the E2 DNA-binding domain from human papillomavirus type 16: Implications for its DNA binding-site selection mechanism. *J. Mol. Biol.* **284**, 1479-1489.

Klug, W. S. and M. R. Cummings. (2000) Chromosome mutations: Variation in chromosome number and arrangement. *Concepts of Genetics*, 6th ed. 262-263.

Lundberg, Jan M. (1995) Tachykinins, sensory nerves, and asthma-an overview. *Can. J. Physiol. Pharmacol.* **73**, 908-914.

Noveral, J. P. and M. M. Grumstein (1995) Tachykinin regulation of airway smooth muscle cell proliferation. *Am. J. Physiol.* **269**, L339-L343.



Human papilloma virus (HPV) infection is an example of a viral disruption of the proliferative balance in cell cycle control. It typically begins by inoculation of an epithelial wound with the viral particles (Ragland et al., 1994). The HPV's double stranded DNA is taken in by a nearby cell and commandeers the host cell's transcriptional machinery to coordinate the expression of viral gene products in a specific spatiotemporal sequence within the differentiating epithelial layer. In this way the early genes are expressed in proliferating undifferentiated keratinocytes (Saunders and Frazer, 1998). The HPV proteins E5, E6, and E7 (See Figure 1.), are responsible for HPV-associated tumor development. The E5 protein inactivates tumor suppressor gene p21 and stimulates human growth factor activity, enhancing cell proliferation and possibly influencing transformation to malignancy (Flaitz and Hicks, 1998; Saunders and Frazer, 1998). Protein E6 binds to the tumor suppressor p53 gene targeting it for destruction (Saunders and Frazer, 1998), as well as binding to the p53 protein which has been referred to as the 'guardian of the genome' (Flaitz and Hicks, 1998). When activated by mutational stimuli, such as ultraviolet- or gamma-irradiation, p53 induces the expression of gene product p21, which inhibits the cell cycle until such time as any DNA damage is repaired. If the DNA damage is too great, then p53 induces apoptosis. Thus, the inactivation of p53 protein by E6 has a dual effect; it removes a proliferative brake from the cell and also leads to genomic instability and mutational inheritance (Saunders and Frazer, 1998). The E7 protein acts as a tumor-promoting agent by binding the tumor-suppressor genes pRb and p107. These tumor suppressors are negative regulators of the viral expression and proliferation-regulating factors, the E2 family. Thus, binding and inactivation of pRb, or p107 leads to the release of cells from negative growth signals and leads to unregulated growth control in the keratinocytes (Saunders and Frazer, 1998; Flaitz and Hicks, 1998). In instances of HPV-infection site transformation to malignancy, the viral DNA is integrated into the host DNA sequence. This integration often causes previously

mentioned E2 gene (which regulates expression of viral genes and viral replication) to be disrupted (Hegde and Androphy, 1998), allowing the E5, E6, and E7 gene products to be produced in a completely deregulated fashion that leads to continued expansion of this malignant cell population. With the cellular controls by p53, p21, and p107 inactivated there is no mechanism to stop the uncontrolled differentiation.

Mosquito saliva of the *Aedes aegypti* mosquito has been found to contain two peptides:

Sialokinin I Asn-Thr-Gly-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH<sub>2</sub>

Sialokinin II Asp-Thr-Gly-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH<sub>2</sub>

(Champagne and Ribeiro, 1994)

They have been identified as being members of the tachykinin peptide family because they contain the carboxyl-terminal sequence Phe-X<sub>aa</sub>-Gly-Leu-Met-NH<sub>2</sub> which is characteristic of this peptide family and is responsible for binding to tachykinin-specific receptors (Champagne and Ribeiro, 1994). These tachykinins, sialokinin I and sialokinin II (SK1 & SK2), have properties similar to tachykinin A and tachykinin B (TKA & TKB) as well as tachykinin substance P (SP) and neurokinin I (NK1) (Champagne and Ribeiro, 1994). Sialokinin I and II (have been proposed to, because of their similarity to TKA, TKB, NK1, and SP) cause a number of physiologic and immune system changes in the mammalian body (Champagne and Ribeiro, 1994). Champagne and Ribeiro (1994) proposed the identity of the two mosquito peptides and proposed their enhancing behavior on mammal neutrophil phagocytosis and macrophage activation, but did not propose an application for these peptides in the cure of unregulated cellular proliferation.

The physiological and immune system changes in mammals caused by tachykinins include vasodilation, vascular permeability, activation of macrophages, activation of neutrophil granulocytes, T-lymphocyte proliferation, monocyte interleukin production, mast cell degranulation

in epithelia, and eosinophil granulocyte degranulation (Lundberg, 1995). Although these characteristics are known of tachykinins, Lundberg has not proposed clinical uses of the mosquito salivary tachykinins, such as sialokinin I and II.

When administered topically or by injection, the tachykinin family can be as effective as when released by their respective production sites (Champagne and Ribeiro, 1994; Lundberg, 1995; Reid et al., 1993; Noveral and Grunstein, 1995). These statements are made about tachykinins in general by the above mentioned sources, but the implications of these functions shared by mosquito salivary tachykinins in their treatment of unregulated cellular proliferation were not recognized.

Cells lining the mosquito larval gut as well as the saliva gland of the water strider have been observed to display endopolyploidy because something appears to inhibit the cell from dividing after its chromosomes have multiplied (Klug and Cummings, 2000). This observation has been made, but no suggestion as to why has been proposed. We propose that this is very possibly because of the action of tachykinins present in the gut of mosquitoes and very probably also in the salivary glands of water striders.

Studies of mammalian tachykinin NK1 receptor antagonist binding have shown that binding and blocking of certain tachykinin receptors by selective receptor antagonists can have an anti-tumor inhibiting activity in the mammalian body (Palma et al., 2000 and U.S. patent #5990125). Simply slowing or inhibiting a tumor is not the solution of the tumor's threat; elimination of the tumor removes the threat (as attested by the number of tumors which physicians surgically remove or destroy by radiation therapy every year), and thus the tachykinin NK1 receptor antagonist treatment does not go far enough. The critical step forward from a tumor inhibitor to tumor-fighting immune response stimulant was not recognized by the previous sources.

## SUMMARY OF THE INVENTION:

Administering mosquito salivary tachykinins, such as sialokinin I and II, topically or by injection to areas of unregulated cellular proliferation “usually referred to as tumors or warts” in the mammalian body will be successful in remediation of the problem area. “Unregulated cellular proliferation” as discussed in the scope of this proposal is undesirable cellular proliferation and differentiation as induced in the HPV example, but not exclusive to HPV tumors and warts alone. “Remediation of the problem area” as defined in this proposal, is the body’s recovery of physiological and genetic control, disappearance of the unregulated cellular growth, and elimination of cancer danger in this tissue.

## DETAILED DESCRIPTION OF THE INVENTION:

It is proposed that if a therapeutically effective amount of mosquito salivary tachykinins were injected into (similar to a mosquito bite and subsequent injection of its saliva), or applied topically to, unregulated mammalian tumor or wart tissue, that the previously mentioned immuno-physiologic mechanisms which they trigger would be induced. It is proposed that mosquito salivary tachykinins themselves inhibit cellular division and that the immuno-physiologic mechanisms which they trigger also would cause the mammalian body to combat the unregulated cellular proliferation. An inflammatory response in the area of injection is expected. This inflammatory response should include activation of alveolar macrophages, neutrophil granulocytes, fibroblast proliferation, T-lymphocyte proliferation, monocyte interleukin production, eosinophil granulocyte degranulation, and mast cell degranulation. Vasodilation and increased vascular permeability in the area of injection is also expected. It is proposed that with these results is also expected a disappearance of the unregulated tissue by apoptosis of the cells in the tumor or wart. In cases of HPV, stimulation of



the host organism's immune system to attack viral protein E6, allows mammalian p53 and p21 to reassume control and the polyp will destroy itself by self-induced cell death. It is proposed that the regeneration of fibroblasts and properly regulated epithelial proliferation necessary for repair of the area disrupted by unregulated cellular proliferation are also expected. It is proposed that as these cell growth regulatory processes transpire, disappearance of visible keratosis and wart structure, and elimination of cancer danger will be observed. In the HPV example, host immune inactivation of viral E5 protein will allow tumor suppressor gene p21 to function and regulate growth factor activity, controlling cell proliferation and stopping possible transformation to malignancy. Host immune attack on HPV E7 protein expression will enable the affected cells to resume tumor suppression through actions of pRb and p107.

The observed result of these proposals will be the expected disappearance of the unregulated tissue structure, or tumor. Once the unregulated proliferation has been suppressed, mosquito salivary tachykinins also regulate epithelial proliferation in areas of epithelial damage inducing healing of the tumor damaged area. Since removal of the tumor's threat is the most desired outcome, mosquito salivary tachykinins offer a minimally intrusive and nearly painless treatment to the menace. The use of invertebrate mosquito salivary tachykinins instead of a receptor antagonist in mammals will function to bind to the mammalian tachykinin receptor sites because of its chemical similarity to mammalian tachykinins TKA, TKB, SP, and NK1 and carry with it the powerful physiological and immune system stimulating abilities of the mosquito tachykinin on the mammalian body. Thus the use of mosquito salivary tachykinins on mammalian tumors will actually stimulate the mammalian body to destroy the tumor rather than simply inhibit its growth as in the case of the previously mentioned NK1 receptor antagonist.

**It is claimed that:**

1. Proteins of the tachykinin family in mosquito saliva, including sialokinin I and II, but not exclusively limited to sialokinin I and II, will inhibit cellular division and induce cells of vertebrate tissues to regain proliferative control in areas of unregulated cellular proliferation.
2. Proteins of the tachykinin family in mosquito saliva will also induce repair of the affected area of the cellular proliferation by immune response and apoptosis of the undesirable growth, but not exclusively limited to these mechanisms.
3. Mosquito salivary tachykinins, such as sialokinin I and II, are therapeutically effective when used singularly or in unison.
4. When mosquito salivary tachykinins, such as sialokinin I and II, are administered topically or by injection (but not exclusively such methods), to regions of unregulated cellular proliferation they will retain their effectiveness as claimed above.
5. Mosquito salivary tachykinins are effective in the mammalian body because of their similarity to mammalian tachykinins which allows them to bind to the mammalian tachykinin receptor and, instead of acting as a tachykinin receptor antagonist, use this advantageous location to perform their immune system and regulatory control stimulatory functions.

## ABSTRACT

Administration of mosquito salivary tachykinins, such as sialokinin I and II, topically or by injection to an area of unregulated cellular proliferation will result in the recovery of cellular proliferative control. The tachykinins cause a number of physiological and immune system changes which induce recovery of proliferative control. These changes include vasodilation, increased vascular permeability, activation of macrophages, activation of neutrophil granulocytes, T-lymphocyte proliferation, monocyte interleukin production, mast cell degranulation in epithelia, and eosinophiles; all of which function to stimulate the body to regain the delicate proliferative balance which has been disturbed by various environmental and viral insults on the body such as UV radiation, ionizing radiation, HPV, and other insults that cause tumors or warts through the same mechanisms.

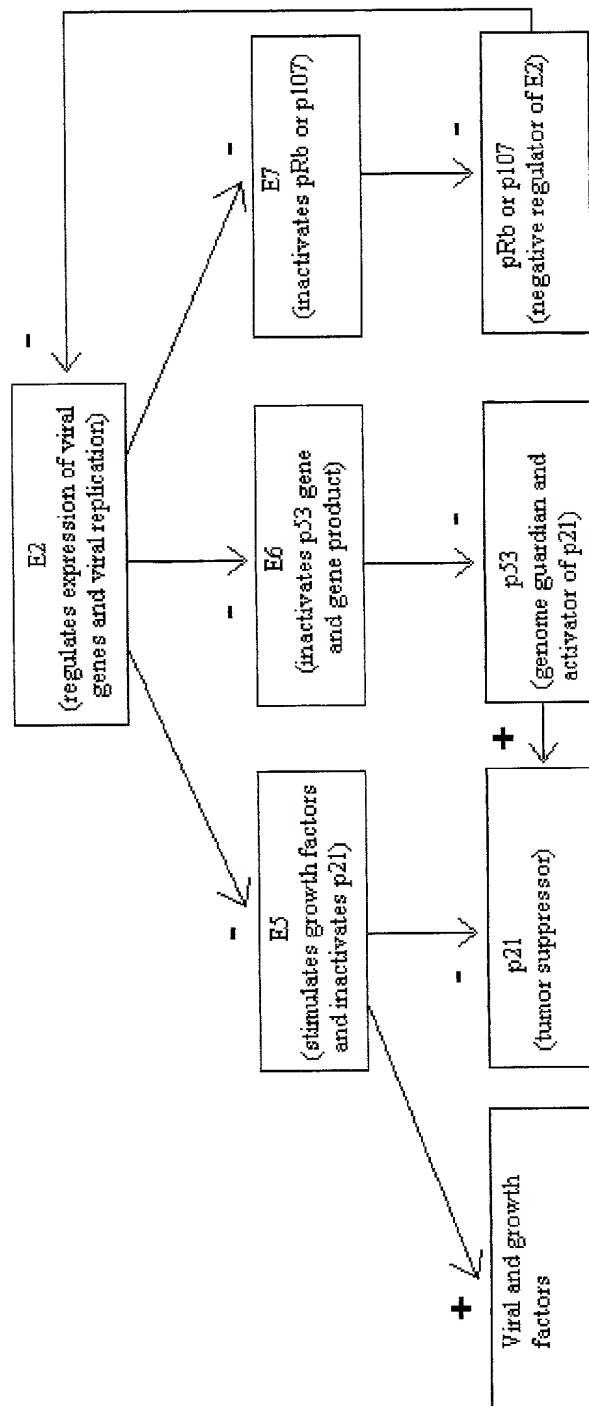


Figure 1. (HPV infection mechanisms)

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<b>DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)</b>  <input checked="" type="checkbox"/> Declaration Submitted with Initial Filing      OR <input type="checkbox"/> Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)	<b>Attorney Docket Number</b>	
	<b>First Named Inventor</b>	Nathan C. Maier
	<b>COMPLETE IF KNOWN</b>	
	<b>Application Number</b>	/
	<b>Filing Date</b>	
	<b>Group Art Unit</b>	
	<b>Examiner Name</b>	

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

The use of mosquito salivary tachykinins to remediate unregulated cellular proliferation

(Title of the Invention)

the specification of which

☒ is attached hereto

OR

☐ was filed on (MM/DD/YYYY)

as United States Application Number or PCT International

Application Number

and was amended on (MM/DD/YYYY)

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

[Page 1 of 2]

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## DECLARATION — Utility or Design Patent Application

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NAME OF SOLE OR FIRST INVENTOR :

☐ A petition has been filed for this unsigned inventor

Given Name **Nathan Christopher**  
(first and middle [if any])

Family Name **Maier**  
or Surname

Inventor's  
Signature

*Nathan Maier*

Date

*Nov. 14/00*

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Country **U.S.A.**

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☐ A petition has been filed for this unsigned inventor

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(first and middle [if any])

Family Name **Jarstfer**  
or Surname

Inventor's  
Signature

*Amiel Jarstfer*

Date

*11-14-00*

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State **TX**

ZIP **75605**

Country **U.S.A.**

☐ Additional inventors are being named on the \_\_\_\_\_ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.